

The University of Chicago

A COMPARISON IN THE FETAL GUINEA
PIG OF THE PATHOGENICITY OF THE
H₃₇ TUBERCLE BACILLUS TO THE
BACILLUS OF CALMETTE-GUERIN

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HYGIENE
AND BACTERIOLOGY

1936

By
IRWIN SAMUEL NEIMAN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
TRANSACTIONS OF THE NATIONAL TUBERCULOSIS ASSOCIATION
Vol. XXXII, 1936



Digitized by the Internet Archive
in 2026

The University of Chicago

A COMPARISON IN THE FETAL GUINEA
PIG OF THE PATHOGENICITY OF THE
H₃₇ TUBERCLE BACILLUS TO THE
BACILLUS OF CALMETTE-GUERIN

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HYGIENE
AND BACTERIOLOGY

1936

By
IRWIN SAMUEL NEIMAN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
TRANSACTIONS OF THE NATIONAL TUBERCULOSIS ASSOCIATION
Vol. XXXII, 1936



A COMPARISON IN THE FETAL GUINEA PIG OF THE PATHOGENICITY OF THE H37 TU- BERCLE BACILLUS TO THE BACILLUS OF CALMETTE-GUERIN*

BY IRWIN SAMUEL NEIMAN

CHICAGO, ILL.

Despite the fact that the *Bacillus* of Calmette-Guerin (BCG) is used in many clinics throughout the world as a vaccine to protect individuals against tuberculosis, it cannot be disputed that doubts as to its harmlessness still exist. While the majority of investigators support the idea that BCG is an attenuated strain of tubercle bacillus incapable of initiating progressive tuberculosis in man or laboratory animals, or of reverting to a virulent form, there are a few contrary reports. Bocchine¹ was able to produce progressive tuberculosis with large doses of BCG. Dreyer and Vollum² believed they caused a reversion to virulence by growing BCG in the depths of a special broth. Petroff and Branch³ recovered a virulent strain from BCG by dissociation. Sassano and Medlar⁴ reported that BCG, grown on the surface of Sauton's medium enriched with normal rabbit serum, became virulent for the rabbit. Although many of these experiments have not been confirmed, the thought remains that if one had a highly susceptible animal subject to rigid experimental control some degree of virulence might be demonstrated for this organism.

Woolpert⁵ has called attention to the living mammalian fetus as an experimental animal favorable for certain types of bacteriologic studies, and we⁶ have reported the reaction of the guinea pig fetus to BCG and to H37. The present paper summarizes our experience to date in this field.

We chose the guinea pig fetus for a study of BCG because the guinea pig is the classic test animal for the tubercle bacillus and because the fetus is inherently sterile and well protected against chance infection from the outside. Another possible advantage is that the fetus may be more

*From the Department of Hygiene and Bacteriology, University of Chicago, Chicago, Illinois.

susceptible than the postnatal animal to the tubercle bacillus, just as it has been found more susceptible to the vaccinia virus⁷ and to the submaxillary gland virus of guinea pigs.⁸

The technic briefly consists of the inoculation of fetuses by needle puncture through the maternal uterine wall and fetal membranes, usually after surgical exposure of the maternal uterus. The fetuses were recovered after a suitable experimental period, either by cesarean section or through spontaneous delivery at term. Most of the fetuses used were 30 to 40 days of age (1 to 10 gms. in weight). Inoculations were made intracerebrally, principally because under these conditions and at this age the fetal head is the part most readily and certainly identified. It is probable also that the guinea pig is more susceptible by this route, as indicated by the work of Feldman.⁹ In the use of this technic a certain degree of contamination of the maternal tissues by the inoculum is inevitable, but in our experience there was no evidence that maternal infection influenced the course of the fetal infection. Although most of the mothers were sacrificed at cesarean section, of those that were permitted to live only one developed gross tuberculosis. (Its fetuses had been inoculated with H37.)

EXPERIMENTS WITH H37

In order to establish a basis for judging the results with BCG a series of fetuses were inoculated with the well-known human strain H37. In a preliminary experiment the virulence of our strain of H37 was demonstrated in postnatal guinea pigs. Of 40 adult animals inoculated subcutaneously with 1 mgm. of the dry bacteria 5 died in less than six weeks. The remainder were extensively tuberculous when sacrificed at the end of seven weeks. Six guinea pigs 4 to 5 days old were inoculated intracerebrally. One received 0.01 mgm., another 0.1 mgm., and 4 were given 1.0 mgm. All of these died of generalized tuberculosis 15 to 28 days later.

Thirty-two fetuses were inoculated intracerebrally with H37 in a dosage ranging from 0.01 to 1.0 mgm. Of these 13 were alive and 11 were dead when removed by cesarean section 5 to 28 days after injection; 6 were spontaneously delivered dead and 2 died shortly after birth (20 and 29 days after inoculation). Gross and microscopic examination was made of all significant tissues and cultures and smears were made of the brains.

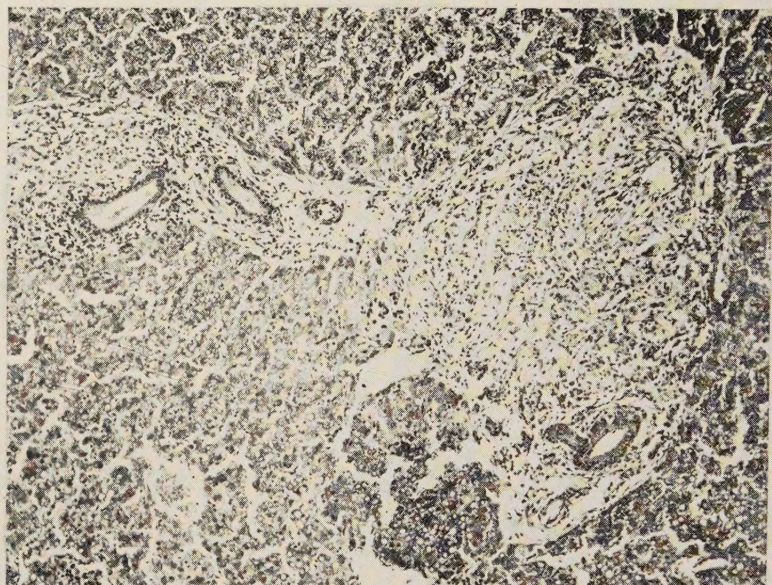
A local meningitis was commonly observed microscopically and occasionally seen grossly. The extent of meningeal involvement varied directly with the dosage and the interval between inoculation and examination. Caseous nodules were discernible grossly in the basal meninges of those fetuses delivered dead or dying soon after birth. An internal hydrocephalus was present in most fetuses, except those receiving 0.01 mgm. of the organisms



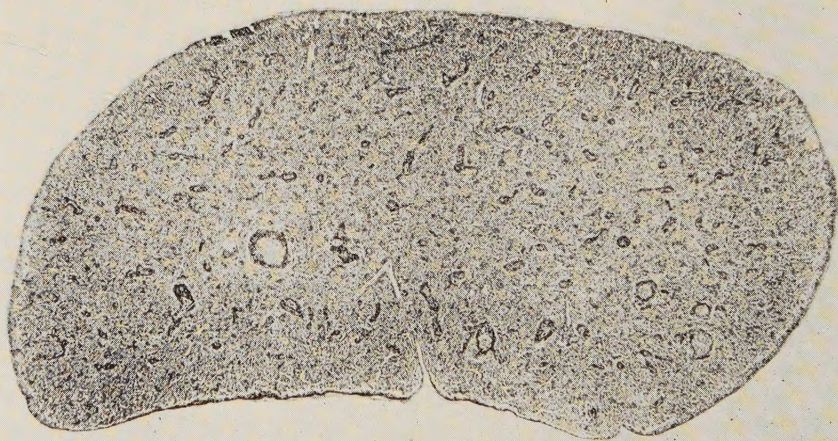
Figure 1. Longitudinal section of fetal brain removed 25 days after inoculation from a fetus injected with 0.01 mgm. of H37. Arrow points to tubercle occluding the foramen of Luschka. X $7\frac{1}{2}$.

Figure 2. Longitudinal section of fetal brain removed 35 days after inoculation from a fetus injected with 0.1 mgm. of BCG. Note tubercle in cerebral tissue. Arrow points to tubercle occluding the foramen of Luschka. X $7\frac{1}{2}$.

Figure 3. Same type of section of fetal brain removed 7 days after inoculation from fetus injected with 0.1 mgm. of BCG. Note moderate enlargement of the lateral ventricle. X 11.



4



5

Figure 4. Section of fetal liver removed 28 days after inoculation with 1 mgm. of BCG. Tubercle surrounds a portal triad. X 125.

Figure 5. Longitudinal section of entire fetal spleen removed 28 days after inoculation with 1 mgm. of BCG. Note the great number of miliary tubercles. X 14.

or examined in the early stages. This may have been due to microscopic tubercles occluding the foramina of Luschka (Fig. 1). In addition, the ventricular walls and brain tissue proper were invaded by tubercle bacilli which set up caseating foci (Fig. 2).

Dissemination of the tubercle bacilli to other organs was followed. About three weeks after inoculation with all dosages, gross tubercle formation was observed in the liver, spleen, and lungs. The liver was found to be the organ first affected and contained bacilli as early as 5 days after intracerebral inoculation, even with the smallest dose. In the spleen the occurrence of the organisms was variable; sometimes they appeared early in the course of the infection and at other times late. The lungs were found to be infected after a period of three weeks in fetuses receiving 0.01 mgm. and in one week in those injected with 0.1 mgm. Occasionally the placenta was found to have miliary tubercles (Fig. 6). In the liver the portal triads were most often the site of tubercle formation (Fig. 4). There was no predilection for any specific structural unit in the lungs and spleen, although in the latter, reactions frequently occurred in or near Malpighian bodies (Fig. 5). Tubercles in the placenta were often in the maternal sinuses.

The microscopic structure of the tubercles in the fetus varied with the age of the tubercles. Very young tubercles were composed chiefly of polymorphonuclear leukocytes with only a few large mononuclears. Tubercles in the later stages of development had caseating centers, invaded and surrounded by large mononuclear cells. Lymphocytes then appeared and formed a collar about the entire area. No giant cells were seen that could be definitely identified as the typical Langhans variety. "Satellite" tubercles were observed in many of the tissues. This is supposed to be indicative of invasiveness of the bacilli, since the "satellites" are obviously (from their cell structure) younger than the central tubercle.

The tubercle bacilli themselves were found principally within large mononuclear cells, although organisms were often seen outside the cells. Occasionally in the early stages of the disease they were lying free in the tissues with practically no reaction about them. They had a granular appearance and were often seen in groups lying parallel to one another (Fig. 7). This last, according to the analysis by Woodruff,¹⁰ indicates growth and multiplication.

EXPERIMENTS WITH BCG

The BCG culture used for most of our experiments was a strain (k) kindly sent us by Dr. W. H. Park. It has been carried on Sauton's liquid medium and on Long's synthetic medium. Of late we have been using

Löwenstein's medium adjusted to a pH of 6.9, to the exclusion of others. In some of the early experiments a strain of BCG received in our laboratory from the late Dr. Calmette through Dr. Florence Seibert was used. The usual precautions were taken to keep BCG from becoming contaminated with H37. BCG cultures were kept in a separate incubator from that in which H37 was grown. Likewise, animals whose fetuses received the former were caged in a different room from those whose fetuses received H37.

In a preliminary experiment (with strain k) each of 9 adult guinea pigs weighing 500 to 900 gms. was injected subcutaneously in the inguinal region with 5 mgms. of the organism. Seven weeks later they were all sacrificed. No pathology suggesting tuberculosis was found except for enlargement of the regional lymph nodes. In another experiment a series of 20 young guinea pigs, 1 to 10 days old, were inoculated intracerebrally with 1 mgm. of BCG. They were killed singly after certain intervals. Acid-fast bacilli were found in smears of the brains from those examined within six weeks after inoculation. After that period they were no longer observed. At no time were tubercles found in any organs other than the brain, except in those animals sacrificed within 2 weeks after inoculation. However, the bacteria in these were intracellular and in large irregular clumps. None of the animals suffered any obvious ill effects due to the inoculum.

The initial fetal experiments with BCG were concerned with relatively large amounts of the inoculum. One and four-tenths to 3.0 mgms. of the dry bacteria (Calmette strain) were injected intracerebrally into fetal guinea pigs of varying ages. Seven fetuses in 3 mothers were inoculated with 3 mgms. of the bacilli from 5 to 7 days before spontaneous delivery. All of the fetuses died of generalized tuberculosis from 1 to 45 days after birth. Ten younger fetuses in 3 mothers were inoculated with 2 mgms. of this bacterium. Four died of generalized tuberculosis at birth. The remaining 6, when examined *in utero* 2 weeks after inoculation, were found to be tuberculous although alive. Three fetuses in one mother were injected with 1.5 mgms. They were removed individually 8, 14 and 20 days after inoculation. The tuberculosis was found to be progressively greater in each fetus, the last being dead when removed. Four fetuses were inoculated with 1.4 mgms. and all were dead of generalized tuberculosis at delivery 31 days after inoculation.

In the remaining fetal experiments with BCG, strain k was used and smaller dosages were employed. In this series 32 fetuses were inoculated with amounts ranging from 0.01 to 1.0 mgm. Only 5 fetuses were inoculated with 0.01 mgm. because this amount was found to cause little pathology in the fetus. Three fetuses receiving this dose were delivered

TABLE 1
Intracerebral inoculation of fetal guinea pigs with 0.1 mgm. BCG

Fetus number*	Age of fetus in days	Time (days) from inoculation to disposition	Gross findings	Microscopic findings	
				Normal tissues	Tissues showing tubercles
175LV	39	7	Lateral ventricles distended	Lung Kidney Placenta	Brain Liver Spleen
155RH	34	7	Lateral ventricles distended	Lung Liver Spleen Placenta	Brain
161RH	42	14	Lateral ventricles distended	Lung Spleen Kidney Placenta	Brain Liver
158RM	33	14	Lateral ventricles distended	Lung Kidney	Brain
736LH	35	21	Lateral ventricles distended	Kidney	Brain Lung Liver Spleen Placenta
736RH	35	21	Lateral ventricles distended	Lung Liver Spleen Kidney Placenta	Brain (smear)
168RH	36	21	Lateral ventricles distended	Lung Liver Spleen Kidney Placenta	Brain (smear)
737RO	37	22	Lateral ventricles distended	Lung Kidney Placenta	Brain Liver Spleen
737LH	37	22	Lateral ventricles distended	Lung Kidney Placenta	Brain Liver Spleen
163RV	34	24	Lateral ventricles distended	Lung Liver Spleen Kidney Placenta	Brain (Granules†)
164RO	35	35	Lateral ventricles distended. Spleen gray. Two lesions one mm. in diameter on kidney.	Kidney	Brain Lung Liver Spleen

*The first three numbers of the fetus number indicate the number of the mother. The letters following refer to the position of the fetus in the uterine horns. Thus:

RH —single fetus in the right horn.

LH —single fetus in the left horn.

RO —the fetus occupying the ovarian end of the right horn.

RV —the fetus occupying the vaginal end of the right horn.

RM —when there are three fetuses in the right horn this indicates the fetus in the middle.

LOM—when there are four fetuses in the left horn this indicates the second fetus from the ovarian end of the horn.

†There were acid-fast granules in the walls of the lateral ventricles. They were intracellular in large macrophages. They appeared to be disintegrating bacteria.

TABLE 2

Intracerebral inoculation of fetal guinea pigs with 1.0 mgm. BCG

Fetus number	Age of fetus in days	Time (days) from inoculation to disposition	Gross findings	Microscopic findings	
				Normal tissues	Tissues showing tubercles
160RH	47	5	Lateral ventricles distended	Lung Spleen Kidney Placenta	Brain Liver
175LO	39	7	Lateral ventricles distended	Lung Spleen Kidney Placenta	Brain Liver
155LH	34	7	Lateral ventricles distended. Caseous nodule in the meninges between cerebrum and cerebellum.	Spleen Kidney Placenta	Brain Liver
161LH	42	14	Lateral ventricles distended. Caseating tubercles 1 mm. in diameter in lung. Liver had necrotic border.	Kidney	Brain Lung Liver Spleen
168LH	36	21	All tissues normal	Brain Lung Kidney Liver Spleen Placenta	
737RV	35	22	All tissues normal	Lung Kidney Placenta	Brain Liver
163LH	34	24	All tissues normal	Lung Liver Spleen Kidney Placenta	
163RO	34	24	All tissues normal	Lung Liver Kidney Placenta	
172RH*	38	28	Lateral ventricles distended. Lungs consolidated. Necrotic areas about 2 mm. in diameter in liver. Spleen gray.		Brain Lung Liver Spleen
164RV†	35	35	Lateral ventricles distended. Liver surfaces showed 1 or 2 necrotic areas about 2 mm. in diameter and 10 to 15 pin-head-size areas. Spleen gray. Kidney hyperemic.		Brain Lung Liver Spleen Kidney

*Lived 11 hours in incubator after cesarean section.

†Killed and autopsied two days after birth.

naturally. One was dead at birth but showed no tuberculous changes; one was sacrificed and necropsied at 6 months of age and found to be normal; the third was killed at 18 months of age and no pathology was observed. Two fetuses examined *in utero* 14 days after inoculation were alive; one was apparently normal in every respect, and in the other only minor local histologic changes were noted.

Twenty-seven fetuses were inoculated with either 0.1 or 1.0 mgm. amounts of BCG. Of these, 2 were killed 2 days after delivery, 2 died immediately after birth, and one died 1 day and another 4 days after delivery. All showed generalized tuberculosis at necropsy, manifested by caseous nodules in the brain and liver and a gray nodular spleen. A single animal which received 0.1 mgm. was killed 18 months after inoculation. No pathology suggestive of tuberculosis was revealed at the necropsy. The 20 remaining fetuses were removed alive by cesarean section from 7 to 28 days after inoculation. The only gross change locally was a distension of the lateral ventricles which was explained, at least in part, by microscopic tubercles occluding the foramina of Luschka. A meningitis was demonstrable microscopically in most of the fetuses. Only with the larger doses and after longer intervals were tubercles found in the brain tissue proper.

The liver was found to be the most frequent site of metastasis. The lungs and spleen often contained bacteria, but the placenta was seldom involved and the kidneys only once. However, dissemination was not as rapid as seen with H37. Those fetuses inoculated with 0.1 mgm. did not show tuberculous involvement of organs other than the brain except after the longer intervals, but those receiving 1.0 mgm. manifested a more rapid spread.

Table 1 lists those fetuses inoculated with 0.1 mgm. of BCG. There were several irregularities noted. Although fetuses 736LH and 736RH were in the same litter and received the same inoculum, one of them manifested involvement of every tissue examined except the kidney, while the other revealed only a relatively few organisms in the brain smear. No explanation suggests itself. The absence of spread from the site of inoculation in fetuses 168RH and 163RV was explained by the fact that in none of the fetuses inoculated with the suspension used for these two animals was there a metastasis from the brain, nor did control cultures of inoculum grow out. Therefore, the assumption was made that these fetuses received few, if any, living organisms. On the whole, with this dosage it was noted that there was spread to other organs after longer periods.

In table 2 are recorded those fetuses inoculated with 1.0 mgm. of BCG. It differs from the preceding table principally in that a great deal more gross pathology was visible in a shorter period and dissemination of the bacteria

TABLE 3

Intracerebral inoculation of fetal guinea pigs with heat-killed tubercle bacilli

Fetus number*	Age of fetus in days	Inoculum	Dose†	Disposition	Time‡	Gross observations	Microscopic findings
701-1	45	BCG	1 mgm.	Died 5 hours after birth	24	Lateral ventricles distended	No tuberculous areas in any tissue. There was evidently an obstruction of the cerebro-spinal canal somewhere, but the process was not found. A smear of the hydrocephalic fluid was negative for acid-fast organisms.
701-2	45	BCG	1 mgm.	Died 10 hours after birth	24	Lateral ventricles distended	Same as above
778-1	28	BCG	1 mgm.	Died 1 hour after birth	33	Lateral ventricles distended	Same as above
778-2	28	BCG	1 mgm.	Killed	180	All tissues normal	All tissues normal
778-3	28	BCG	1 mgm.	Killed	270	All tissues normal	All tissues normal
663-1	30	BCG	1 mgm.	Killed	180	All tissues normal	All tissues normal
663-2	30	BCG	1 mgm.	Killed	270	All tissues normal	All tissues normal
632-1	37	BCG	2 mgms.	Alive to date (one and one-half years after inoculation)			
632-2	37	BCG	2 mgms.	Alive to date (one and one-half years after inoculation)			
846LO	31	H37	0.1 mgm.	Cesarean section	6	All tissues normal	Localized meningitis. Tubercle bacilli present. All other tissues normal.
846RH	31	H37	1 mgm.	Cesarean section	6	All tissues normal	Localized meningitis. Tubercle bacilli present. All other tissues normal.
840-1	37	H37	0.1 mgm.	Killed	450	All tissues normal	All tissues normal
840-2	37	H37	0.01 mgm.	Killed	450	All tissues normal	All tissues normal

*1 or 2 after the maternal number indicates that the fetuses were delivered but cannot be recognized.

†Milligrams of dead bacilli, killed by heating for 1 hour at 60° C.

‡Time in days from inoculation to disposition.

was more rapid, as would be expected. Fetuses 168LH, 163LH and 163RO were inoculated with the faulty suspension mentioned above and may reasonably be left out of consideration.

Table 3 lists those fetuses inoculated with heat-killed bacteria. Of 13 so inoculated all were delivered alive and apparently normal, but 3 died soon after birth. When the latter were necropsied an internal hydrocephalus was found with a localized meningitis in which no acid-fast bacteria were demonstrable. All the other organs were essentially normal. No tuberculous pathology was seen in the other 10 when they were killed and examined at the times indicated.

The tubercles found in the fetuses receiving BCG had the same cellular structure and showed the phenomenon of "satellite" tubercles as described for those produced by H37. The acid-fast bacilli had the same microscopic appearance as H37 and were extracellular as well as intracellular. In some cases they were in the large mononuclear cells in such great numbers that it was impossible to interpret their presence except on the basis of their multiplying in the cytoplasm of these cells.

Recovery of BCG on artificial media was accomplished several times but with difficulty. After various methods of concentration had been tried, the technic of Corper and Uyei,¹¹ utilizing 6 per cent H_2SO_4 , was found satisfactory. Before inoculation the BCG colonies were predominantly of the "crater" type (Fig. 8). The organisms recovered from the brain were mixtures of these and heaped-up colonies with broad flat bases and glistening raised centers (Fig. 10, Fig. 11, and Fig. 12). One mgm. of the recovered bacteria was injected into other fetuses. From the brains of the latter animals organisms were recovered which formed colonies even more distinctive than those first recovered (Fig. 13). However, when the recovered bacteria were subcultured on fresh media they tended to revert, for the most part, to the original "crater" colonies (Fig. 14 and Fig. 15).

The recovered bacteria are being tested for any possible change in virulence, but this phase of the work is incomplete at the present time. In two instances it was possible to infect fetuses by the intracerebral route with treated tuberculous fetal brains (Fig. 16). These fetuses exhibited wide involvement of the visceral organs. In both cases only one direct transfer was made.

DISCUSSION

In this study we have compared the reaction of the fetal guinea pig to BCG with its reaction to the H37 strain of tubercle bacillus. The results of others, working with adult guinea pigs, and our own control inoculations in postnatal animals afford also a basis for comparison of the reactions of fetal guinea pigs with those of mature animals. It should be noted that

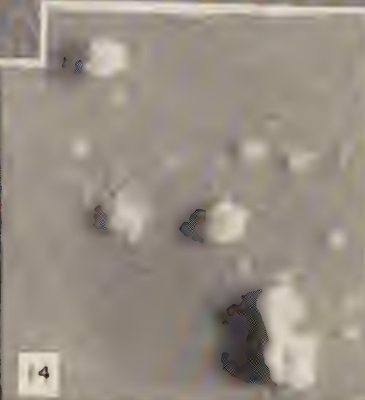
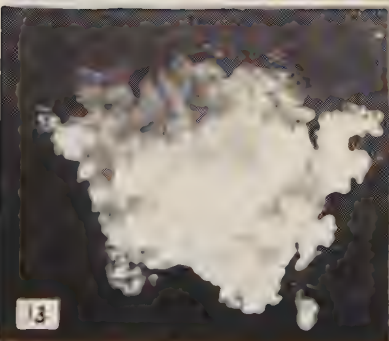
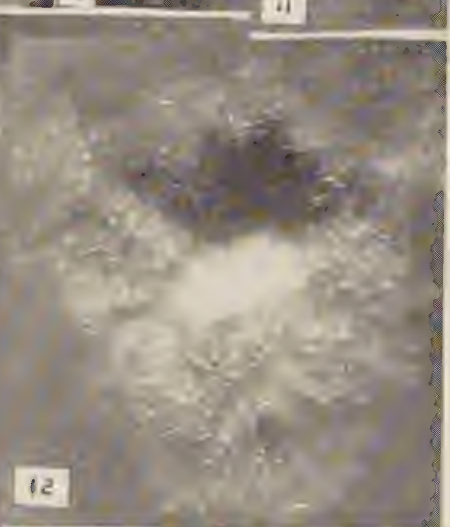
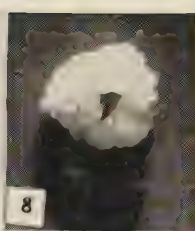
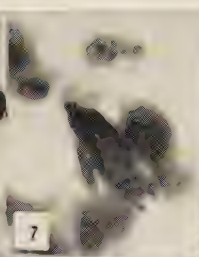
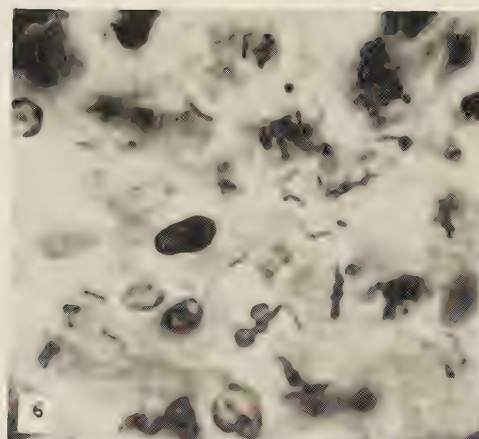


Figure 6. Early tubercle in placenta removed 25 days after fetus had been inoculated with 0.1 mgm. of H37. This tubercle was closely associated with an older tubercle. X 1500.

Figure 7. Photomicrograph of large mononuclear cell containing many acid-fast bacteria lying more or less parallel to one another. From brain of fetus inoculated with 0.1 mgm. of BCG. X 1000.

Figure 8. "Crater" colony formed by original BCG. (Park strain k.) X 6.

Figure 9. Another type of colony formed by original BCG. (Park strain k.) X 6.

Figure 10. A number of colony types that were recovered from brain of fetus injected with original BCG. X 3.

Figures 11 and 12. Higher magnification of two of the colonies with spreading bases. X 16.

Figure 13. Type of colony recovered from brain of fetus inoculated with BCG shown in Figures 10, 11 and 12. X 16.

Figures 14 and 15. Types of colonies obtained after 2 or 3 subcultures on artificial media of BCG recovered from fetuses. X 4.

Figure 16. "Y" formation of acid-fast bacilli in a tubercle at the base of the brain of a fetus inoculated with 0.1 cc. of a suspension of brain material from a tuberculous fetus. X 2500.

we used two different strains of BCG, although most of the work was done with one strain, and that we observed the usual precautions in keeping separate our BCG and H37 cultures and animals. No special medium was used in an attempt to enhance any innate virulence of BCG. The histologic and bacteriologic evidence obtained indicates that both H37 and BCG are capable of multiplying in fetal guinea pig tissues and of initiating progressive disease processes which may lead to widespread tuberculous involvement and death of the fetus. However, it was clearly evident that BCG is less virulent than H37, since larger doses or longer experimental periods were required to produce with BCG changes comparable to those seen in H37 infections. Amounts of BCG less than 0.1 mgm. appeared incapable of producing progressive disease, whereas 0.01 ingm. amounts of H37 were lethal, and probably much smaller amounts would have been effective.

From these results it may be inferred that the fetal guinea pig, intracerebrally inoculated, constitutes a highly susceptible animal for work with the tubercle bacillus. It is probable also that the younger fetuses are much more susceptible than the older fetuses, although we do not have comparative data to present in this connection. It should be remembered that the morphologic development of the fetus proceeds at a very rapid rate. At 30 days of age, according to Ibsen,¹² the guinea pig fetus weighs only a little over 1 gm., at 35 days the weight averages about 4 gms., and at 40 days about 9 gms. The weight of the newborn animal averages around 75 gms. It is not surprising, therefore, that fetuses inoculated at 30 to 35 days of age should manifest reactions qualitatively and quantitatively different from those of the postnatal animal, nor is it surprising, we believe, that BCG exhibits a definite degree of virulence for these fetuses of an animal species itself highly susceptible to virulent tubercle bacilli. What the implications of these findings may be with respect to the whole problem of the use of BCG in man we prefer to leave for further consideration. We wish to emphasize primarily two points: that the guinea pig fetus is susceptible to tuberculous infection, and that it constitutes a highly favorable animal for virulence studies because of its susceptibility and its freedom from contamination with extraneous organisms.

SUMMARY

Guinea pig fetuses were inoculated intracerebrally with graded doses of BCG and the H37 strain of tubercle bacillus.

Following varying incubation periods, some of the fetuses were removed by cesarean section, others were permitted to be delivered naturally.

Both types of tubercle bacillus were found capable of spreading from

the site of inoculation and of causing progressive tuberculous disease processes and death of the fetus. However, H37 spread with greater facility and was effective in smaller amounts.

H37 was recovered from the fetal tissues on artificial media with ease, but BCG was recovered only with difficulty. The recovered strains of BCG differed somewhat in colony morphology from the strains inoculated.

Virulence studies on the recovered strains are incomplete, but the organisms appear to be as pathogenic for the fetal guinea pig as the original strain. In two instances we have infected fetuses with treated tuberculous tissue taken from other fetuses that had been inoculated with BCG.

On the basis of our experience we conclude that the fetal guinea pig is an animal highly favorable for this type of experimentation, and that a certain degree of virulence can be demonstrated for BCG in the fetal guinea pig.

REFERENCES

1. Bocchine, A.: Ricerche sulla innocuita e sulla efficacia del vaccino B.C.G. nei conigli trattati per via endovenosa. *Pediatria*, 1928, 36: 169.
2. Dreyer, G., and Vollum, R. L.: Mutation and pathogenicity experiments with BCG. *Lancet*, 1931, 1: 9.
3. Petroff, S. A., and Branch, A.: Bacillus Calmette-Guerin (BCG) in prophylactic immunization in children. *Canadian Medical Association Journal*, 1928, 13: 581.
4. Sassano, K. T., and Medlar, E. M.: Studies on the Calmette-Guerin strain of tubercle bacillus. I. The effect *in vitro* of environment on the virulence of BCG. *Amer. Rev. Tuberc.*, 1931, 23: 215.
5. Woolpert, O. C.: Direct bacteriological experimentation on the living mammalian fetus. *Amer. Jour. Path.*, 1936, 12: 141.
6. Neiman, I. S., and Woolpert, O. C.: Intracerebral inoculation of fetal guinea pigs with Bacille Calmette-Guerin and the H37 strain of tubercle bacillus. *Amer. Jour. Path.*, 1936, 12: 153.
7. Stritar, J., and Hudson, N. P.: Susceptibility of the guinea pig fetus to vaccinia. *Amer. Jour. Path.*, 1936, 12: 165.
8. Markham, F. S., and Hudson, N. P.: Susceptibility of the guinea pig fetus to the submaxillary gland virus of guinea pigs. *Amer. Jour. Path.*, 1936, 12: 175.
9. Feldman, W. H.: A study of the pathogenicity of the Bacillus of Calmette-Guerin (B.C.G.) *Amer. Jour. Path.*, 1932, 8: 755.
10. Woodruff, C. E.: A free growth period of tubercle bacilli in the guinea pig omentum as related to the hypersensitive state. *Amer. Jour. Path.*, 1934, 10: 739.
11. Corper, H. J., and Uyei, N.: The isolation of tubercle bacilli from contaminated tuberculous materials. *Amer. Rev. Tuberc.*, 1927, 16: 299.
12. Ibsen, H. L.: Prenatal growth in guinea pigs, with special reference to environmental factors affecting weight at birth. *Jour. Exp. Zool.*, 1928, 51: 51.

